



APPENDIX G

Diagnosis of Renal Cancer by Molecular Urinalysis

Claus F. Eisenberger, Mark Schoenberg, Cheryl Enger, Steven Hortopan, Shefali Shah, Nan-Haw Chow, Fray F. Marshall, David Sidransky

Background: Organ-confined renal malignancies can be cured in the majority of patients, whereas more extensive lesions have a poor prognosis. We sought to develop a noninvasive test for renal cancer detection based on a novel molecular approach. **Methods:** Matched urine and serum DNA samples were obtained before surgery from 30 patients with clinically organ-confined solid renal masses (25 with malignant tumors and five with tumors of low malignant potential) and were subjected to microsatellite analysis. Serum samples and urine samples obtained from 16 individuals without clinical evidence of genitourinary malignancy served as controls. **Results:** Nineteen (76%) of the 25 patients with malignant tumors were found to have one or more microsatellite DNA alterations in their urine specimen, and 15 (60%) were found to have alterations in their serum DNA by microsatellite analysis. In every case, the microsatellite changes in urine or serum were identical to those found in the primary tumor. Three of five patients with tumors of low malignant potential were found to have DNA alterations in their urine, but none displayed alterations in their serum. Moreover, microsatellite alterations were not identified in either the urine or the serum samples from normal control subjects and patients with hematuria due to nephrolithiasis (renal stones). **Conclusion:** These data suggest that microsatellite DNA analysis of urine specimens provides a potentially valuable tool for the early detection of resectable kidney cancer. Furthermore, microsatellite analysis of serum samples reveals evidence of circulating tumor-specific DNA in approximately half of these patients and may reflect the propensity of these tumors to spread to distant sites at an early stage. [J Natl Cancer Inst 1999; 91:2028-32]

Approximately 30 000 new cases of malignant renal cancer are diagnosed each year in the United States, and nearly 12 000 individuals succumb to the effects of metastatic disease annually (1). Although organ-confined renal carcinoma can be cured by radical nephrectomy, carcinoma that extends beyond the confines of the renal capsule is associated with substantial morbidity and a high rate of cancer-specific mortality (2). The application of conventional adjuvant therapies to patients with advanced disease has been disappointing because of the resistance of most malignant renal tumors to standard chemotherapy and radiotherapy (2).

The specific clinical signs and symptoms of malignant renal disease are not usually helpful in making an early diagnosis. The classic triad of pain, hematuria, and a palpable flank mass is encountered in only 10% of patients and is usually associated with the presence of advanced disease (3). The widespread use of noninvasive axial imaging (e.g., computed tomography or magnetic resonance imaging) and diagnostic ultrasound has resulted in the incidental discovery of an increasing number of small asymptomatic renal tumors. Estimates suggest that as many as two thirds of organ-confined tumors are identified by serendipity (4). Nevertheless, 50% of patients with renal cancers are not curable by surgical resection at the time of presentation. Consequently, the development of a reliable, noninvasive method for the early detec-

Affiliations of authors: C. F. Eisenberger, S. Hortopan, N.-H. Chow (Department of Urology, James Buchanan Brady Urological Institute), M. Schoenberg, F. F. Marshall (Department of Urology, James Buchanan Brady Urological Institute, and The Johns Hopkins Oncology Center), C. Enger (The Johns Hopkins Oncology Center), S. Shah (Department of Otolaryngology—Head and Neck Surgery, Division of Head and Neck Cancer Research), D. Sidransky (Department of Otolaryngology—Head and Neck Surgery, Division of Head and Neck Cancer Research, and The Johns Hopkins Oncology Center), The Johns Hopkins Medical Institutions, The Johns Hopkins University School of Medicine, Baltimore, MD.

Correspondence to: David Sidransky, M.D., The Johns Hopkins University School of Medicine, Otolaryngology—Head and Neck Surgery, Head and Neck Cancer Research, 818 Ross Research Bldg., 720 Rutland Ave., Baltimore, MD 21205-2196 (e-mail: DSIDRANS@JHMI.EDU).

See "Notes" following "References."

© Oxford University Press

tion of kidney cancer could represent an important clinical advance in the management of this patient population.

Microsatellite analysis is a polymerase chain reaction (PCR)-based technique that permits the detection of cancer-specific DNA alterations (loss of heterozygosity [LOH] and microsatellite instability) in neoplastic tissue. Recent application of this approach to the evaluation of body fluids has shown that squamous cell carcinoma of the aerodigestive tract and bladder cancer can be detected through the analysis of saliva and urine, respectively (5-7). Since the renal parenchyma is highly vascular and lies in close physical proximity to the renal collecting system and since the tubular epithelium from which most malignant renal neoplasms arise contributes directly to urine formation, we hypothesized that DNA alterations characteristic of malignancy could be identified by microsatellite analysis of either serum or voided urine specimens obtained from patients with renal malignancies. To test this hypothesis, we studied, by use of microsatellite analysis, preoperative urine and serum specimens obtained from patients with a variety of renal neoplasms.

MATERIALS AND METHODS

Sample collection and DNA isolation. After we obtained written informed consent from 30 patients with a renal lesion, samples of peripheral blood and urine were collected before surgical intervention. The study was approved by the Institutional Review Board of The Johns Hopkins Hospital. Neoplastic kidney tissue was obtained immediately after surgical resection and stored at -80°C. Eight control samples were obtained from patients with nephrolithiasis (renal stones) and eight from individuals without a history of genitourinary disease (total = 16). Tumor tissue was microdissected as previously described (8). DNA was obtained from peripheral lymphocytes, serum, and tumor samples by digestion with the use of proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany) in the presence of sodium dodecyl sulfate at 48°C overnight, followed by phenol-chloroform extraction and ethanol precipitation.

Microsatellite analysis. Based on high rates of informativity and known patterns of LOH and microsatellite instability in renal cancer, 28 microsatellite markers (Research Genetics, Huntsville, AL) were identified for use in this series of experiments. Microsatellite markers (and their chromosomal locations) are as follows: D1S251 (1p), HTPO (2p), D3S1317 (3p), D3S587 (3p), D3S1560 (3p), D3S1289 (3p), D3S1286 (3p), D3S1038 (3p), D4S243 (4p), FGA(4) (4q), CSF (5q), ACTBP2 (5p), D8S348 (8q), D8S307 (8p), D9S747 (9p), D9S242 (9p), IFN α (9p), D9S162 (9p), D11S488 (11q), THO (11p), vWA (12p), D13S802 (13q), MJD (14q), D17S695 (17p), D17S654 (17p),

D18S51 (18q), MBP (18q), and D21S1245 (21q). Primer sequences and locations were obtained from the Genome Database (The Johns Hopkins University, Baltimore, MD). One primer from each pair was end-labeled with [γ -³²P]adenosine triphosphate (Amersham Life Science Inc., Arlington Heights, IL) with the use of bacteriophage T4-poly nucleotide kinase (Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD). Genomic DNA (50 ng) was subjected to 35 PCR cycles at a denaturing temperature of 95°C for 1 minute, followed by varying annealing temperatures (52°C-60°C, depending on the primer sequence) for 1 minute, an extension step at 72°C for 1 minute, and a final extension step at 72°C for 5 minutes by use of a thermocycler (Hybaid, Teddington, U.K.). PCR products were then separated in denaturing 7% polyacrylamide-urea-formamide gels, followed by autoradiography for 12-36 hours on X-omat film (Eastman Kodak Co., Rochester, NY) (9). LOH was determined by a comparison of the intensity of the allelic bands from nonmalignant (lymphocyte) DNA with that of the allelic bands from the target sample (from tumor, urine, or serum). A reduction in the intensity of one allele in the target sample of more than 50% (30% in serum as a result of a greater dilution with normal DNA), as assessed by two independent observers (C. F. Eisenberger and D. Sidransky), was considered to represent LOH and the presence of new "shifted" alleles (appearance of new bands) as microsatellite instability. Clinical data were obtained from the patient charts. Every marker alteration (LOH or microsatellite instability) was confirmed by reamplification of the starting material and independent verification by two separate observers.

Statistical analysis. The sensitivity and specificity of marker alterations in urine and serum were calculated as follows: sensitivity = number of positive tests/number of cancer cases, and specificity = number of negative tests/number of cases without cancer. The proportion of patients showing a microsatellite alteration was compared between tumor stages with the use of Fisher's exact test. *P* values reported are two-sided.

RESULTS

We tested 28 microsatellite markers from 20 chromosomal regions in paired urine and serum specimens from case patients with cancer and control subjects. Nineteen (76%) of 25 patients with malignant tumors of the kidney (Table 1) were found to have one or more microsatellite alterations (microsatellite instability and LOH combined) in the urine DNA, and 15 (60%) of these 25 patients were found to have at least one alteration (microsatellite instability and LOH combined) in the serum DNA (Fig. 1, Table 1). In each case, the identical genetic alteration was also found in the corresponding DNA from the tumor sample. Three (60%) of the five patients with tumors of low or negligible malignant potential (one angiomyolipoma and two oncocytomas) displayed LOH at one or more markers in the urine that also matched the pattern of

LOH found in the corresponding tumor (Table 1). None of the eight healthy subjects and none of the eight patients with nephrolithiasis displayed alterations in the urine or serum. Thus, detection of microsatellite alterations in the urine had a sensitivity of 73% (22 of 30 patients in this study) for identifying patients with a renal mass and 100% specificity. The corresponding sensitivity and specificity for serum were 50% (15 of 30) and 100%, respectively.

One patient with transitional cell carcinoma (TCC) of the renal pelvis was included in the present analysis (#1290, Table 1). DNA changes were readily detectable in the urine and serum samples obtained from this patient preoperatively. The finding of readily identifiable LOH in the urine of this patient is consistent with previously published results obtained from studies of microsatellite analysis in patients with TCC of the bladder (6,7). Unlike patients with bladder cancer (Linn CJ, Sidransky D: unpublished data), however, this patient with cancer of the renal pelvis displayed serum abnormalities as well (Table 1).

There was no association between evidence of either microsatellite instability or LOH in serum and the stage of tumor (Table 2). Fifteen (60%) of 25 patients treated for malignant renal neoplasms in this study had evidence of either LOH or microsatellite instability in serum samples obtained preoperatively. Of these 15 patients, three had tumors of stage T1, eight had tumors of stage T2, and four had tumors of stage T3, and there was no difference in serum detection between any of these tumor stages (*P* = .39, Fisher's exact test). Similarly, the urine samples were positive in 19 patients, including four patients with stage T1 tumor, 11 with stage T2 tumor, and four with stage T3 tumor. Again, there was no association between stage of tumor and evidence of microsatellite alterations in urine specimens (*P* = .39, Fisher's exact test). Patients with lesions of low malignant potential failed to show evidence of serum alterations in this analysis.

DISCUSSION

Pathologic stage of disease predicts an individual patient's clinical outcome more profoundly than any other currently available marker after surgical treatment for a malignant renal tumor. Radical nephrectomy remains the primary mode of therapy for kidney cancers, although le-

Table 1. Samples showing loss of heterozygosity (LOH) and/or microsatellite instability in the tumor, urine, and serum

No.	Pathology*	Age, y	pTNM†	Grade‡	Symptoms/history§	LOH, tumor/ urine/serum	Microsatellite instability, tumor/urine/ serum
1430	RCC, clear cell	51	T2N0MX	II-III	Hematuria	6/0/0	2/0/0
1410	RCC, chromophobe	52	T2N0MX	II-III	Microscopic hematuria	6/1/0	1/0/0
1345	RCC, clear cell	65	T3bN0M1	III	Metastasis (lung, subcutaneous)	2/0/0	0/0/0
1353	RCC, clear cell	72	T3aNXXMX	III	None	2/1/1	0/0/0
1383	RCC, clear cell	65	T3aNXXMX	II	Discomfort	4/1/1	0/0/0
1270	RCC, clear cell	74	T2NXXMX	II	Glomerulosclerosis	5/1/1	1/1/1
1317	RCC, clear cell	58	T2NXXMX	II	Pain, CIS of glans, cholelithiasis	13/8/1	0/0/0
1450	RCC, clear cell	33	T2NXXMX	I	Hematuria/pain	4/0/0	1/1/0
1461	RCC, clear cell	70	T1NXXMX	I-II	None	4/2/1	1/1/1
1463	RCC, clear cell	61	T2NXXMX	II	Hematuria	5/2/0	0/0/0
1494	RCC, clear cell	75	T2NXXMX	II	Recurrent UTI, hematuria	7/4/1	0/0/0
1499	RCC, clear cell	67	T3bN1MX	II-III	Discomfort/mass	4/3/1	0/0/0
1523	RCC, clear cell	60	T2N0MX	II	None	6/1/1	0/0/0
1524	RCC, clear cell	46	T2NXXMX	II-III	None	1/0/0	0/0/0
1540	RCC, clear cell	45	T2NXXMX	I	None	2/1/1	1/1/1
1560	RCC, clear cell	59	T2NXXMX	I	None	2/0/1	0/0/0
1575	RCC, clear cell	60	T1N0MX	I-II	None	2/2/0	1/0/0
1549	RCC, clear cell	61	T2N0MX	II	None, renal pelvis involved	4/0/0	0/0/0
1613	RCC, clear cell	74	T2N0MX	II	None	3/1/1	0/0/0
1634	RCC, clear cell	67	T1N0MX	I	None	3/2/1	0/0/0
1552	RCC, hypernephroid	69	T3NXXMX	III	None	5/3/2	0/0/0
1290	TCC, renal pelvis	76	T1NXXMX	III	Hematuria/positive cytologic specimen	13/8/3	1/1/0
1452	RCC, papillary	70	T2NXXMX	III	None, collecting ducts involved	20/3/0	0/0/0
1551	RCC, papillary	63	T2N0MX	II	None	4/0/0	0/0/0
1550	RCC, papillary	54	T2N0MX	II	None	11/3/2	0/0/0
1500	Angiomyolipoma	63	4 cm		None	5/2/0	1/0/0
1522	Metanephric nephroma	68	4 cm		None	1/0/0	1/0/0
1458	Oncocytoma	77	2 cm		Hematuria/pain	1/1/0	0/0/0
1640	Oncocytoma	74	2.7 cm		None	1/1/0	0/0/0
1614	Oncocytoma	63	4 cm		None	1/0/0	0/0/0

*RCC = renal cell carcinoma; TCC = transitional cell carcinoma.

†American Joint Committee on Cancer staging. pTNM: p = pathologic stage; T = tumor size; N = node status; M = metastatic status.

‡American Joint Committee on Cancer.

§CIS = carcinoma *in situ*; UTI = urinary tract infection.

||Numbers under LOH and microsatellite instability columns indicate positive microsatellite markers in tumor, urine, and serum DNA. In total, 28 markers were tested by microsatellite analysis in each clinical sample. All samples are from patients with malignant tumors, except those from patients 1500, 1522, 1458, 1640, and 1614 whose tumors have low or negligible malignant potential.

Table 2. Microsatellite analysis of clinical samples by stage of tumor at time of surgery

Sample	Stage of tumor*			P†
	T1 (n = 4)	T2 (n = 16)	T3 (n = 5)	
Urine	4 (100)	11 (69)	4 (80)	.39
Serum	3 (75)	8 (50)	4 (80)	.39

*Number (%) with loss of heterozygosity or microsatellite instability in sample.

†Two-sided, Fisher's exact test.

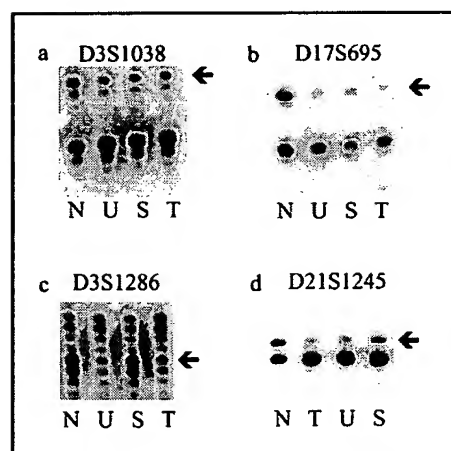


Fig. 1. Microsatellite analysis of clinical samples from patients with renal cancer. Loss of the upper alleles indicating loss of heterozygosity in the urine (U), serum (S), and tumor (T) in patients 1523 (a), 1290 (b), and 1540 (d). Loss of the bottom allele is shown only in urine and tumor but not in serum in patient 1499 (c). Microsatellite markers are depicted over each panel. N = normal lymphocyte DNA.

sions extending beyond the kidney are substantially more difficult to cure than are those limited to the confines of the renal capsule. Although noninvasive imaging has clearly improved clinical staging of renal tumors, neither ultrasound nor computed tomography is sufficiently inexpensive or accessible to be used for widespread early-detection programs.

Advances in basic research have shed light on some events that putatively con-

tribute to the development of renal neoplasia. Detailed studies of pathology (10) have underscored the morphologic heterogeneity of renal cancers. Genetic studies employing a variety of technologies (11-13) have shown that renal cancers are characterized by specific chromosomal abnormalities, the most common of which include subchromosomal losses on 3p in sporadic clear cell carcinomas, aneuploidy of chromosomes 7 and 17 in papillary renal tumors, and small deletions on chromosomal arm 1p in oncocytomas and on distal 1q in collecting duct carcinomas. Elegant familial studies of von Hippel-Lindau (VHL) disease have led to the identification of the gene putatively responsible for that disease located at 3p25 (14). Additional work (15) has shown that LOH at the VHL locus may be characteristic of most sporadic renal cancers. However, these advances in basic research have not yet translated into the development of reliable diagnostic markers of renal cancer.

The central clinical problem facing surgeons and oncologists who care for patients with renal cancer is that this cancer is unresponsive to conventional systemic adjuvant therapies, unlike other genitourinary cancers for which successful adjuvant therapies have been developed (i.e., cis-platinum-based chemotherapeutic regimens for bladder cancer and nonseminomatous testicular neoplasms) (16,17). Radioresistance is also characteristic of renal tumors, leaving surgery as the sole, consistently successful form of therapy. Surgical removal of the kidney has a limited role in the treatment of patients with advanced disease and is often not curative in patients with tumors that extend beyond Gerota's fascia or that involve regional lymphatics. Although prospective trials have not yet confirmed the value of early renal cancer detection, stage-specific survival data suggest that, if renal neoplasms could be consistently detected at the organ-confined stage, the disease-specific survival rate could be expected to increase. It is interesting to note that a recent retrospective autopsy series identified renal cancer as one of the most common undiagnosed tumors that contributed to death in U.S. patients who underwent postmortem examination (18).

The ease with which microsatellite analysis can be performed on a variety of DNA sources continues to increase. The molecular diagnosis of renal cancer by urinalysis is remarkable in that specimens from all patients contained a virtual absence of cellular material or only scant cellular fragments. Further enrichment of neoplastic cell (or cell fragment) populations in the urine with antibodies to cancer-specific antigens such as MN/CA9 protein (19) may further increase the sensitivity of microsatellite analysis. Evolving knowledge of the genetic changes that drive kidney cancer progression and the ability to distinguish malignant and benign tumors of the kidney will also lead to further improvements (20). We have demonstrated the ability to perform multiple analyses using a new high-throughput microcapillary array that can markedly enhance the potential clinical utility of this molecular diagnostic approach (21). Translation of this type of technology to the clinical laboratory may hold the key to broader application and eventual use of microsatellite-based cancer diagnostics.

Urologic malignancies have well-studied patterns of anatomic spread, the best characterized of which are testis and prostate cancers (3). Both of these tumor types are known for their propensity to metastasize by lymphatic routes. In contrast, renal cancer probably spreads by a combination of lymphatic and hematogenous mechanisms, a fact underscored by the finding that circulating cancer cells have been readily extracted from the blood of patients with various stages of malignant renal disease (22). The finding of serum microsatellite alterations in greater than 50% of the patients studied for this report probably reflects the hematogenous mechanism by which certain renal tumors spread. A recent report (23) confirmed a similar frequency of microsatellite alterations in the serum of patients with clear cell renal tumors. While it is not yet possible to say to what extent patterns of microsatellite alterations are related to the malignant and metastatic potential of individual renal tumors, further study of these tumors with different panels of markers may reveal patterns of LOH and microsatellite instability with prognostic as well as diagnostic potential. Coupled with high-throughput technologies, genome-wide search strategies, and the opportunity to study a larger population of patients in a multi-institutional venue, microsatellite analysis may permit the identification of patients with early and potentially resectable disease. Microsatellite alterations in serum predicted a poor prognosis in patients with head and neck cancer (24) and may also identify kidney cancer patients at risk for disease progression for whom experimental adjuvant therapies may be beneficial.

We have demonstrated that microsatellite analysis of urine can frequently detect the presence of malignancy in patients with clinically organ-confined renal cancer. Patients with renal lesions of lower malignant potential, such as oncocytomas, demonstrated DNA alterations in matched urine samples but not in preoperative serum samples. Individuals with nephrolithiasis and control subjects without genitourinary complaints or symptoms did not have positive microsatellite analyses in this study. Larger multicenter trials utilizing this assay for the diagnosis and potential staging of renal cancer patients are warranted to determine the ultimate clinical utility of this molecular approach.

REFERENCES

- (1) Cancer statistics. 1999. *CA Cancer J Clin* 1999;49:8-31, 1.
- (2) Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. *N Engl J Med* 1996;335:865-75.
- (3) Belldgrun A, deKernion JB. Renal tumors. Chapt. 76. In: Walsh PC, Retik AA, Vaughan ED, Wein AJ, editors. *Campbell's urology*. 7th ed. Philadelphia (PA): Saunders; 1998. p. 2283-6.
- (4) Gudbjartsson T, Einarsson GV, Magnusson J. A population-based analysis of survival and incidental diagnosing of renal cell carcinoma patients in Iceland, 1971-1990. *Scand J Urol Nephrol* 1996;30:451-5.
- (5) Spafford MF, Reed AL, Xu L, Koch W, Westra WH, Califano JA, et al. Detection of head and neck squamous cell carcinoma in saliva using microsatellite analysis [abstract]. *Proc Am Assoc Cancer Res* 1998;39:100.
- (6) Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, et al. Molecular detection of primary bladder cancer by microsatellite analysis. *Science* 1996;271:659-62.
- (7) Steiner G, Schoenberg MP, Linn JF, Mao L, Sidransky D. Detection of bladder cancer recurrence by microsatellite analysis of urine. *Nat Med* 1997;3:621-4.
- (8) Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1995;332:429-35.
- (9) Litt M, Hauge X, Sharma V. Shadow bands seen when typing polymorphic dinucleotide repeats: some causes and cures. *Biotechniques* 1993;15:280-4.
- (10) Thoenes W, Storkel S, Rumpelt HJ. Histopathology and classification of renal cell tumors (adenomas, oncocytomas and carcinomas). The basic cytological and histopathological elements and their use for diagnostics. *Pathol Res Pract* 1986;181:125-43.
- (11) Zbar B, Brauch H, Talmadge C, Linehan M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature* 1987;327:721-4.
- (12) Hughson MD, Johnson LD, Silva FG, Kovacs G. Nonpapillary and papillary renal cell carcinoma: a cytogenetic and phenotypic study. *Mod Pathol* 1993;6:449-56.
- (13) Polascik TJ, Cairns P, Epstein JI, Fuzesi L, Ro JY, Marshall FF, et al. Distal nephron renal tumors: microsatellite allelotyping. *Cancer Res* 1996;56:1892-5.
- (14) Latif F, Tory K, Gnarr J, Yao M, Duh FM, Orcutt ML, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;260:1317-20.
- (15) Gnarr JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, et al. Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet* 1994;7:85-90.
- (16) Kaufman DS, Shipley WU, Griffin PP, Heney NM, Althausen AF, Efrid JT. Selective bladder preservation by combination treatment of invasive bladder cancer. *N Engl J Med* 1993;329:1377-82.
- (17) Einhorn LH, Donohue J. Cis-diamminedichloro-

- roplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 1977;87:293-8.
- (18) Burton EC, Troxclair DA, Newman WP 3rd. Autopsy diagnoses of malignant neoplasms: how often are clinical diagnoses incorrect? *JAMA* 1998;280:1245-8.
- (19) Liao SY, Aurelio ON, Jan K, Zavada J, Stanbridge EJ. Identification of the MN/CA9 protein as a reliable diagnostic biomarker of clear cell carcinoma of the kidney. *Cancer Res* 1997; 57:2827-31.
- (20) Steiner G, Sidransky D. Molecular differential diagnosis of renal carcinoma: from microscopes to microsatellites. *Am J Pathol* 1996; 149:1791-5.
- (21) Sidransky D. Nucleic acid-based methods for the detection of cancer. *Science* 1997;278: 1054-8.
- (22) Pontes JE, Pescatori E, Connelly R, Hashimura T, Tubbs R. Circulating cancer cells in renal-cell carcinoma. *Prog Clin Biol Res* 1990;348: 1-12.
- (23) Goessl C, Heicappell R, Munker R, Anker P, Stroun M, Krause H, et al. Microsatellite analysis of plasma DNA from patients with clear cell renal carcinoma. *Cancer Res* 1998; 58:4728-32.
- (24) Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. *Nat Med* 1996;2:1035-7.

NOTES

C. F. Eisenberger and M. Schoenberg contributed equally to the completion of this study.

Supported by Public Health Service grant P01CA77664-02 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from the Deutsche Forschungsgemeinschaft (to C. F. Eisenberger); by grant 96-78 from the American Cancer Society (to M. Schoenberg); and by a grant from the National Science Council, Taiwan, ROC (to N.-H. Chow).

Manuscript received July 16, 1999; revised September 28, 1999; accepted October 1, 1999.